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MELANIN IN THE LONGNOSE GAR, *LEPISOSTEUS OSSEUS*

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ABSTRACT

Examination was made of the melanophores of three Lepisosteus osseus (longnose gar), one dark, one intermediate, and one normal in pigmentation. Melanophore counts were made per mm^2 from anterior, mid, and posterior areas of both the dorsal and ventral surfaces of each of the three fish. The data were tested with a four-factor analysis of variance. By microscopic examination the melanophores were classified as to branching in the sample areas. Neither number of melanophores nor degree of branching explained divergence in pigmentation. However, divergence was attributed to the melanosome color and concentration in the melanophores.

INTRODUCTION

The ability to change color has been observed for numerous cyclostomes, fishes, amphibians and reptiles through movements of pigments within certain integumentary cells. The Roman naturalist and writer Pliny (23-79 A.D.), according to Brown (1946), described color changes of the dying mullet fish. These changes are the function of melanophores, special pigment cells located in the skin or in certain deeper tissues. Melanophores, though non-motile themselves, possess the ability to bring about redistribution of their pigment granules so as to influence the general coloration of the animal. Pigment granules that are concentrated into small balls (punctate) contribute little to the gross coloration of the organism, whereas their dispersion covering a larger surface (stellate) results in imparting a tint to the animal. This mechanism of color change (concentration and dispersion of pigment) is referred to as physiological color change. Melanophores may also influence the coloration of an animal through their accumulation or production of pigment granules, melanosomes, and their loss or destruction of pigment granules, or by differences in total numbers of pigment cells. This mode of action is termed morphological color change.

The melanophore was once thought to be an ameboid cell; contraction of whose processes resulted in a concentration of the pigment mass into a small sphere, and whose extensive pseudopodial production resulted in a broad dispersal of the pigments. Now it has been concluded that the melanophore has a permanent arborized form, and that it is the melanosomes which concentrate or disperse. Spaeth (1913), and others, using the light microscope reported observing branches of melanophores whose pigment was in the punctate condition. This observation was confirmed by Matthews (1931), and Perkins (1932) using the light microscope and by Bikle et al. (1961) using electron microscopy.

The rate of physiological color change is limited by the rates of mechanical response of the effector organ and of its controlling mechanism. There is, therefore, great variation in such rates. For example, a squid is able to carry out maximum color change in a matter of seconds, as is also the fish Holocentrus sp., whereas from one to several hours are needed by many crustaceans and the catfish Ameriurus (Ictalurus) sp.; and days are required for comparable maximum changes in flatfishes, eels, elasmobranchs, and amphibians according to Brown (1936).

A number of methods have been utilized in the measurement of physiological color changes and these have been reviewed by Parker (1943a). The measurements are based on time intervals of sufficient

brevity to assure that morphological changes would not have influenced the results significantly. None of the methods permits complete differentiation between influences which are the result of morphological color changes and those which are purely physiological. One group of methods employs the gross changes in color of the animal as an index of the extent of dispersion of the dark pigments. This may involve a visual determination in which the animal is described as being light, dark or intermediate, or in which subjective grades of variation between known extremes are estimated and expressed numerically as in Brown's work (1946). Some of the subjective aspects have been removed by Wright (1955) and by Novales (1959) using a method employing photometric determination of the fraction of the incident light reflected from a unit area of skin surface. Smith (1936) utilized the relative amounts of light transmitted by isolated fish scales.

A second group of methods was based not on the gross light absorptive changes in the skin, but rather on the changes in the chromatophores themselves. One of these methods is the measurement of the dispersion of melanosomes by the measurement of the diameter changes caused by the dispersion of melanosomes within the melanophores. This method was employed for fish by Spaeth (1916) and by Brown (1936). Another method, and one extensively adopted, describes the melanophore morphology as punctate, punctostellate, stellate, reticulostellate

and reticulate. This system has the advantage that quick inspection can yield numerical values adequate for many comparative purposes (Slome, 1928 and Matsumoto, 1954).

Morphological color changes involve actual changes in quantity of pigment within skin. In the normal adaptive color changes physiological and morphological changes may result in both an increase in the number of melanosomes within each pigment cell and in the number of functional melanophores per unit area of skin (Odiorne, 1933). Quantitative studies of morphological color changes have involved two types of techniques: (1) the determination of changes in the number of functional melanophores per unit area of surface, and (2) the determination of changes in the total pigment content of the fish by chemical extraction and colorimetric determination of pigment quantity in the extracts. This subject was reviewed by Sumner (1940).

A close functional relationship between physiological and morphological color changes was noted by both Keeble et al. (1905) and Babak (1913). According to Babak's law, the maintained punctate condition of melanosomes within a melanophore seems usually to be correlated with a reduction in quantity of melanosomes, and, conversely, pigment dispersion (stellate) is associated with pigment production. Then pigment formation or destruction results from the state of dispersion of the pigment, or both physiological and morphological color changes are affected in a parallel manner by the same controlling

manner by the same controlling mechanisms.

The problem of melanism in fish was brought to my attention by Dr. William S. Woolcott. The Aquatic Division of Biology at the Virginia Institute for Scientific Research (VISR) which he heads had been studying the effects of a power station's thermal discharge on organisms in the James River. During the three year study they have collected eleven dark longnose gar (Lepisosteus osseus) and two intermediate pigmented gar out of a total collection population of 256 gar.

A literature search revealed that dark longnose gar have been observed previously in another location. Goff (1935) picked up a melanistic specimen in Lake Harris, Lake County Florida.

The objectives of this study were to determine the bases for melanism in the specimens of longnose gar collected by Woolcott and to describe pigmentation patterns in normal pigmented as well as melanistic longnose gar.

MATERIALS AND METHODS

Three frozen longnose gar were selected from the collection at VISR which were collected by electrofishing near Bremo Bluff, Virginia, in September 1973. Specimens included one darkly (total length 71 cm, weight 1,134 g), one intermediately (total length 61 cm, weight 610 g), and one normally pigmented gar (total length 63 cm, weight 539 g). All three were mature males.

Sections of skin approximatly 1 cm^2 were taken from anterior, mid, and posterior areas of both the dorsal and ventral surfaces of each of the three fish. The tissue was fixed overnight in 10% formalin and then washed in 70% ethyl alcohol for thirty minutes and transferred to 95% ethyl alcohol for one hour and then transferred to fresh 95% ethyl alcohol and left overnight. The skin was cleared and stored in methyl salicylate.

Each section of skin was examined with a stereo microscope at 80x and counts were made of the melanophores of both the inner (dermis below the scale) and outer (dermis and epidermis above the scale) surfaces of each piece. The counts were made in 1/25 mm^2 increments with the aid of an ocular grid to ascertain the population per mm^2. Eight areas selected at random were examined for each surface of each

piece of skin, and the means of these counts were determined.

A four-factor analysis of variance was computed for the cell counts, with (A) the color of the fish, (B) area (anterior vs. mid vs. posterior), (C) skin surfaces (inner vs. outer), and (D) side (dorsal vs. ventral) as the main effects. On those interactions found to be significant at the 95% level orthogonal comparisons were done in accord with Steel and Torris (1960).

Using a modification of Matsumoto's (1954) classification, each section of skin was classified as to which type of melanophore occured most: punctate (no branching), stellate (slight branching with central concentration of pigment), reticulostellate (moderate branching with central concentration of pigment), and reticulate (diffuse branching with no central concentration of pigment). The concentration and color of the melanosomes within melanophores from all sections were determined by microscopic observation.

RESULTS AND DISCUSSION

Light is the most important environmental factor influencing the state of the melanophore system. It involves the eyes, central nervous system, and various types of efferent pathways, nervous, hormonal or both. The importance is manifested in the immediate cessation, or great change in character of color changes on the blinding of a fish.

Color changes which are controlled by way of the eyes are secondary responses, in contrast with primary responses which are either the direct action of light on the melanophores, or the influences of light operating on the melanophores reflexively through extraocular receptor mechanisms. Secondary color changes occur through a wide range of light intensities. These changes are determined by the values of the ratios of the amount of light directly striking the eye from above to the amount of light reflected from the background on which the fish resides. According to Brown (1936), on an illuminated black background where the ratio is larger, the fish becomes dark and on an illuminated white background where the ratio is small, the animal becomes pale, irrespective of the total illumination. There is, therefore, a direct correlation between the value of the ratio of incident

light to reflected light reaching the eye and the degree of pigment dispersion (Sumner and Keys 1929) and the amount of melanin formed in melanophores (Sumner, 1940). An example of this is reported by Osborn (1940) who indicated functional melanophores formed on the normally unpigmented ventral surface of flounders after they were placed in tanks illuminated from below, or blinded and illuminated from above. The character of this secondary color response appears in general better devloped in fish which are bottom dwellers.

The primary response is due to the direct influence of light on the melanophores. Brown (1946) studied this in melanophores after denervation of the melanophores by nerve transection, in melanophores which are not under innervation, and in fish embryos whose melanophores have not yet come under secondary control. It also has been shown by Brown (1946) that if the minnow Ericymba sp. is placed on a black background, illumination does not influence coloration as long as there is more than 1.75 foot candles. At a lower illumination than this, the diameters of the melanophores are a linear function of the logarithm of the incident light down to 0.00053 foot candles.

Fishes show a spectrum of mechanisms of coordination of melanophore activity ranging from systems involving primitive hormonal control through those in which both nerves and hormones cooperate, to those largely dominated by direct innervation (Brown, 1946). In

fish, melanophores are the means by which responses to light, darkness and to white and black backgrounds occur. The mechanism of response changes from primary to secondary color responses during early development is exhibited by the work of Duspiva (1931) and Neill (1940) with fishes Perca sp. and Salmo sp. However, other species appear not to pass through a phase of primary response but to have initially the secondary type, which is exhibited by the work of Wayman (1924) and Parker (1941) for Fundulus sp., Tomita (1936) for Scyllium sp., and Parker (1936b) for Mustelus sp. Some fishes showing secondary color responses will revert to primary responses if blinded (Tomita, 1936).

Secondary color responses of fish are dependent on the eyes, involving nervous pathways to the central nervous system. This response is accomplished by the endocrine glands affecting the melanophores through hormones or by way of efferent nervous pathways directly to the melanophores where chemical mediators are liberated by the nerve terminations. Another situation can exist where hormonal and nervous mechanisms work together. Parker (1943b) divides the fishes into three groups on the basis of the degree to which direct innervation of melanophores is found. Dineuronic melanophores possess double innervation with separate dispersing and concentration fibers. Mononeuronic melanophores possess single innervation in which the activity is always

pigment concentrating. Aneuronic melanophores possess no innervation; their secondary responses are solely activited by blood and lymph-borne chemical factors.

Teleost fishes as investigated by Parker (1941) possess dineuronic melanophores. This is exemplified in Parker's work by one type of innervation nerve fiber which concentrated pigment when the appropriate nerve loci were stimulated in the central nervous system. The extent of blanching paralleled the area of skin innervated by the nerve, and upon cessation of the stimulation the area of skin was no longer blanched. This action of the concentrating fibers is mediated through adrenalin-like material which diffused away from the nerve terminations.

The presence of pigment dispersing nerve fibers was found in the teleost fish Fundulus sp. (Parker, 1936a). The caudal radiating nerve was cut and darkening occurred and then faded, then the nerve was cut again and darkening occurred again. This behavior led Parker (1936a) to believe that the melanophore response observed was due to a restimulation of the dispersing nerve fibers that had been transected and stimulated by the first incision. An activation of the melanin-dispersing fibers in the catfish tail was demonstrated by Parker and Rosenblueth (1941) by use of electrical stimulation. Other teleost fish have been shown to have similar melanin-dispersing fibers: Holocentrus sp. according to Parker (1937), Paradilurus

sp. according to Matsushita (1938), Pterophyllum sp. according to Fries (1942).

There is further evidence both morphological and physiological for dual innervation of teleost melanophores. Ballowitz (1893) demonstrated that the melanophores of perch receive nerve terminations from more than one fiber. The work of Fujii and Novales (1969), using the electron microscope, showed two types of presynaptic terminals for the melanophores of the teleost Lebistes sp., one containing vesicles 500 Å in diameter and the other 1000 Å in diameter. A study was made of the response of the melanophores at the edges of denervated caudal bands of Fundulus sp. by Mills (1932). A study was made by Abramowitz (1936) of melanophores near the regenerating front of nerve fibers in the course of reinnervation of denervated bands as the fish darken on black backgrounds and lighten on white ones. These works give evidence that many melanophores located in these regenerating fronts possess only one type of fiber, either concentrating or dispersing, but not both as under normal conditions. This is indicated because some melanophores show rapid pigment concentration and very slow dispersion; others show the reverse. Studies of the influence of drugs on the melanophores of Phoxinus sp. by Giersberg (1930) and Fundulus sp. by Smith (1931) support the dual innervation.

The nerve dispersing fibers exert action on the melanophores

through the mediation of acetylcholine. Acetylcholine is known to cause dispersion of the melanin of fishes when they are eserinized to prevent rapid destruction. According to Parker (1940) a bioassay of the acetylcholine content of the skin of a dark adapted catfish, Ameiurus (Ictalurus) sp., and snakefish, Ophiocephalus sp., showed the presence of 0.078 gamma/g of skin of acetylcholine. This is approximately the concentration of acetylcholine found when injected into the body fluids of eserinized fish was effective upon melanophore dispersion. Some investigators, Abe et al. (1969) and Healy and Ross (1966) found acetylcholine to be a weak melanin-dispersing agent in teleosts.

The mononeuronic fish that have been investigated are the dog fishes Mustelus sp. and Squalus sp. According to Parker (1936b) if a transverse cut is made in the pectoral fin of a dogfish of intermediate tint a light band is produced distal to the point of the cut. Such light bands may be viewed after they have redarkened or may be reduced by electrical stimulation according to Parker (1935). These facts point to a nerve supply to the melanophore whose function it is to induce pigment concentration and not pigment dispersion.

Blood born agents typically supplement the nervous system in the secondary responses of the melanophores of fishes to light stimuli. In the more primitive fishes such as the cyclostomes and the elasmo-

branchs, however, hormones alone are the agents involved; therefore, they possess aneuronic pigment cells. Among these hormones is the pigment dispersing principle from the posterior lobe of the pituitary, the B-substance of Hogben (1936) or melanocyte stimulating hormone (MSH) of Zondek (1932). Lundstrom (1932) observed that hypophysectomized Mustelus sp. become and remain pale. The fish may be darkened again by injection of extract of the posterior lobe. A similar role of the posterior lobe has been demonstrated for other elasmobranchs, Raja sp. and Scyllium sp., according to Hogben (1936) and the cyclostome Lampetra sp., according to Young (1935). Eddy and Strahan (1968) found that implantation of the pineal complex into the ammocoete larve of Geotria sp. causes localized pallor and that intraperitoneal injection of melatonin into this lamprey also causes blanching.

According to Hogben (1936), in Scyllium sp. and Raja sp. there is evidence of a second neurohumor from the pituitary that acts as a pigment concentrating agent. The state of the melanophores in these fishes is determined by the ratio of MSH to melanophore concentrating hormone (MCH) present in the blood at any given time, and this ratio is controlled by visual stimuli. These stimuli through differential, dorsoventral, retinal stimulation, result in different rates of secretion of the two hormones, thus giving a bihumoral control.

As there were no living specimens available for physiological examinations in the present study, only morphological evidence was considered. The mean values for the melanophore counts can be seen in Table 1. A four-factor analysis of variance was computed on the melanophore counts with significance at the 95% level (Table 2). The only significant main effect by orthogonal comparisons was the sides (dorsal vs. ventral) factor, which was not involved in any interaction. There was a significantly greater number of melanophores per mm^2 for the dorsal ($\bar{x}$ 217) than for the ventral ($\bar{x}$ 8) side of each gar.

Variance ratios (Table 2) for area (F=101.3) by skin surface (F=29.4) were found to be significant. Orthogonal comparisons indicated a difference in skin surfaces which varied over areas (Figure 1). The mean number (Table 1) of melanophores per mm^2 for the inner ($\bar{x}$ 134) was significantly greater than that for the outer surface ($\bar{x}$ 112) for the anterior areas. There were, however, no significant differences in the number of melanophores per mm^2 in the mid ($\bar{x}$ 118 inner and $\bar{x}$ 122 outer) and posterior areas ($\bar{x}$ 98 inner and $\bar{x}$ 92 outer).

Variance ratios (Table 2) for area (F=101.3) by color of fish (F=42.4) were found to be significant. Orthogonal comparisons indicated a difference in the effect of area for the three gar involved (Figure 2). For the dark gar there were no significant differences among sample areas on the combined dorsal and ventral surfaces in

the numbers of melanophores per mm^2. The mean numbers (Table 1) of melanophores per mm^2 for the areas were anterior, $\bar{x}$ 109, mid, $\bar{x}$ 105, and posterior, $\bar{x}$ 101. The intermediate and normal gar showed no significant differences between the anterior and mid areas. The mean number of melanophores per mm^2 for the intermediate gar was $\bar{x}$ 126 anteriorly and $\bar{x}$ 118 medially, and for the normal gar it was $\bar{x}$ 134 anteriorly and $\bar{x}$ 136 medially. However, the posterior areas exhibited a large discrepancy from the other two areas for both gar. The mean number of melanophores per mm^2 for the intermediate gar ($\bar{x}$ 82) was signigicantly different from the mean for the normal ($\bar{x}$ 103).

No four or three-factor interaction was significant (Table 2). The variance ratios ranged from F=0.9 to F=2.0, which were not signigicant by orthogonal comparisons.

The results of the melanophore classification can be seen in Table 3. There are some differences in the dispersion of melanosomes in the dorsal melanophores. The divergence is greatest when comparing the two darker gar with the normally pigmented one. In both of the darker gar there is greater melanosome dispersion (mostly reticulostellate to reticulate), especially in the posterior region (mostly reticulate in the dark gar and reticulostellate in the intermediate gar) and to some degree in the mid region (stellate and reticulostellate in the dark gar and mostly reticulostellate in the intermediate gar),

as compared to the normally pigmented gar (mostly stellate and punctate in the mid areas and stellate in the posterior areas of the normal gar). The inner skin surface in the dark and intermediate gar has greater dispersion of their melanosomes (reticulostellate to reticulate) than the inner skin surface of the normal gar (stellate). The ventral surfaces of all the gar, however, showed no or little divergence in melanosome dispersion (mostly reticulostellate).

It was usual to find more than one melanophore dispersion pattern on a scale of a gar. Figure 3 shows the mid-dorsal outer skin surface of a scale from the normal gar. The most obvious melanophores are the reticulostellate ones which become very densly packed together along the edge of the scale. Less obvious is a band of punctate melanophores in the mid scale region which are probably mixed with small stellate melanophores. Scales on the mid dorsal line usually were side by side with differing melanophore dispersion patterns. An example would be one scale with mostly stellate melanophores and another next to it with punctate, reticulostellate and reticulate melanophores. Micro- and macromelanophores also were present on each scale. By observation the macromelanophores were approximately 10 times larger than the micro. Both the micro- and macromelanophores did not necessarily conform to the type of dispersion pattern of the dominate type of melanophore of a particular scale.

The typical melanophore types dissected off whole scales can be seen in the figures. Figure 4 shows a typical group of punctate melanophores from the mid dorsal outer skin surface of the dark gar. Figure 5 shows a stellate melanophore from the anterior dorsal outer skin surface of the dark gar, and Figure 6 shows two stellate melanophores from the mid dorsal outer skin surface of the same gar. Figure 7 shows a reticulostellate melanophore from the anterior dorsal inner skin surface of normal gar, and Figure 3 is a scale from the outer skin surface of the same gar. Figure 8, reticulostellate melanophores from the mid dorsal outer skin surface of the dark gar, shows how complex these cells can become. Figure 9 shows a reticulate melanophore from the anterior dorsal outer skin surface of the intermediate colored gar. Notice that in this pigment cell there is no central concentration of melanosomes.

There are several relationships that can be drawn from statistical analysis of melanophore counts and the dispersion of melanosomes in the melanophores. There was a significantly greater number of melanophores for the dorsal ($\bar{x}$ 217 per mm^2) than for the ventral surface ($\bar{x}$ 8 per mm^2) of each gar, and there was greater melanosome dispersion (reticulostellate) on the ventral surfaces than on the dorsal surface (stellate) of each gar (Table 2). As the dark gar does not have a greater concentration of melanophores per mm^2, numbers of

melanophores do not explain the difference in color.

Microscopic examination of the melanophores of the three gar showed that the more melanistic the fish, the greater was the concentration of melanosomes and darker their color in the melanophores. Figures 4, 5, and 6 show the high concentration of black melanosomes in punctate and stellate melanophores of the dark gar. Figures 8 and 10 show the dense concentration of black melanosomes in reticulostellate melanophores of the dark gar. Figrue 9 shows the moderate brown melanosome concentration within the melanophores of the intermediate gar. Figures 3 and 7 show the less dense concentration of the yellow melanosomes in the melanophores of the normal gar.

In the present study eleven (4.3%) of the total number of 256 gar collected were dark and two (0.8%) of the total collected were intermediate in pigmentation. Figure 11 shows the difference in pigmentation between the dark and normally pigmented gar. Of the dark gar collected the weight range was from 560 g to 2,542 g (Table 3). The longest dark gar was 88.3 cm and the shortest 56.0 cm. The weight and total length range indicates that the melanism was not due to size and age, assuming size is a function of age. And, since at least one dark gar was collected during the various calender seasons (Table 4), it would appear that the melanism was not a seasonal change condititon.

The dark gar collected were of both sexes (Table 3). Out of the

eleven dark gar collected five were male and four were female, three of which had eggs. The almost equal distribution of sexes and the presence of various stages of the reproductive cycle indicate that melanism was not due to sex or stage of reproductive cycle.

The dark gar were collected in and out of the thermal plume caused by the power plant at Bremo Bluff. Six of the dark gar were collected in heated water and five were collected in water of ambient temperature. It would seem that normal and dark gar were similarly distributed with regard to the heated water.

The evidence presented in this study indicates that differences in pigmentation are due to different amounts and color of melanosomes rather than to physiologically induced differences in the dispersion of the melanosomes in the melanophores. Excluding major physiological influences, every phase of the development and function of pigment cells is under the control of specific genes within the cells and tissues. Genes within pigment cells control the size, shape, protein structure, and number of melanosomes produced; the amount of melanin produced; and the polymerization of melanin, and probably through this polymerization of melanin the color of the granules, whether black, brown, or yellow (Breathnach, 1969). In view of the fact that the concentration and color of melanosomes is basically genetic in origin, and as melanistic gar have been found in varying conditions

relating to such things as age, sex, and environment, the evidence presented here might suggest the variations in pigmentation of the three gar are primarily genetic in origin.

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TABLE 1: Means of melanophore counts per mm^2 for the skin surfaces of L. osseus.

Main effect	A_1, A_2, A_3 by B_1, B_2, B_3	A_1, A_2, A_3 by C_1, C_2	A_1, A_2, A_3 by D_1, D_2	B_1, B_2, B_3 by C_1, C_2	B_1, B_2, B_3 by D_1, D_2	C_1, C_2 by D_1, D_2
A_1 104.9	A_1, B_1 108.6	A_1, C_1 107.1	A_1, D_1 200.0	B_1, C_1 111.8	B_1, D_1 235.9	C_1, D_1 201.0
A_2 108.7	A_1, B_2 105.3	A_1, C_2 102.7	A_1, D_2 9.7	B_1, C_2 134.2	B_1, D_2 7.9	C_1, D_2 14.4
A_3 124.6	A_1, B_3 100.7	A_2, C_1 99.9	A_2, D_1 208.5	B_2, C_1 121.5	B_2, D_1 232.9	C_2, D_1 233.1
B_1 123.0	A_2, B_1 126.4	A_2, C_2 115.4	A_2, D_2 6.8	B_2, C_2 118.4	B_2, D_2 7.1	C_2, D_2 0.9
B_2 120.0	A_2, B_2 118.1	A_3, C_1 116.2	A_3, D_1 242.7	B_3, C_1 91.9	B_3, D_1 182.4	
B_3 95.2	A_2, B_3 81.5	A_3, C_2 132.9	A_3, D_2 6.4	B_3, C_2 98.4	B_3, D_2 7.9	
C_1 108.4	A_3, B_1 133.8					
C_2 117.0	A_3, B_2 136.5					
D_1 217.1	A_3, B_3 103.3					
D_2 8.3						

A_1=Dark gar, A_2=Intermediate gar, A_3=Normal gar

B_1=Anterior, B_2=Mid, B_3=Posterior

C_1=Outer, C_2=Inner

D_1=Dorsal, D_2=Ventral

TABLE 2: Analysis of variance summary table for the melanophore counts per mm^2 of L. osseus.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio
A	2	21065.4	10532.7	42.4*
B	2	50327.9	25163.9	101.3*
C	1	7303.3	7303.3	29.4*
D	1	111436.7	111436.7	448.6*
AB	4	46104.8	11526.2	46.4*
AC	2	650.8	325.4	1.3
AD	2	725.4	362.7	1.5
BC	2	15610.0	7805.1	31.4*
BD	2	645.9	322.9	1.3
CD	1	499.3	499.3	2.0
ABC	4	1410.8	352.7	1.4
ABD	4	998.4	249.6	1.0
ACD	2	476.9	238.4	0.9
BCD	2	521.7	260.8	1.1
ABCD	4	1351.3	337.8	1.4
ERROR	252	62600.0	248.4	
TOTAL	287	259120.7		

A=Color of Fish, B=Anterior vs. Mid vs. Posterior Area, C=Inner vs. Outer Dermal layers, D=Dorsal vs. Ventral Sides, * Significant Variance

TABLE 3: Distribution of predominant melanophore types on specimens of L. osseus of varying degrees of melanism.

Side		Anterior	Mid	Posterior
Normal				
Dorsal	outer skin	stellate	stellate	stellate
	inner skin	stellate	punctate	stellate
Ventral	outer skin	reticulostellate	reticulostellate	reticulostellate
	inner skin	------	------	------
Intermediate				
Dorsal	outer skin	stellate	punctate	stellate
	inner skin	stellate	reticulostellate	reticulate
Ventral	outer skin	reticulostellate	reticulostellate	reticulostellate
	inner skin	reticulostellate	reticulostellate	reticulostellate
Dark				
Dorsal	outer skin	stellate	stellate	reticulostellate
	inner skin	stellate	stellate	reticulate
Ventral	outer skin	reticulostellate	reticulostellate	reticulate
	inner skin	reticulostellate	reticulostellate	reticulate

TABLE 4: Total length, weight and collection dates of the black and intermediatly pigmented L. osseus collected from the James River.

Collect. Da.	Water Temp.	Sex	Tot. lg. (cm)	Wt. (g)
Dark				
Sept., 1972	ambient	-	63.9	727
Aug.	ambient	M	74.9	1,182
Aug.	ambient	M	91.1	2,318
Sept., 1973	ambient	M	71.0	1,134
Sept.	heated	M	61.0	610
Oct.	heated	F	86.5	2,110
Nov.	heated	M	56.0	560
Jan., 1974	heated	-	72.5	1,728
Apr.	heated	F	88.9	2,352
Apr.	ambient	F	65.8	708
May	heated	F	88.3	2,452
Intermediate				
May, 1974	heated	F	67.8	652
May	ambient	F	71.0	570

FIGURE 1. Histogram showing the average number of melanophores per mm^2 in the anterior, mid and posterior areas of the inner and outer skin surfaces of the gar.

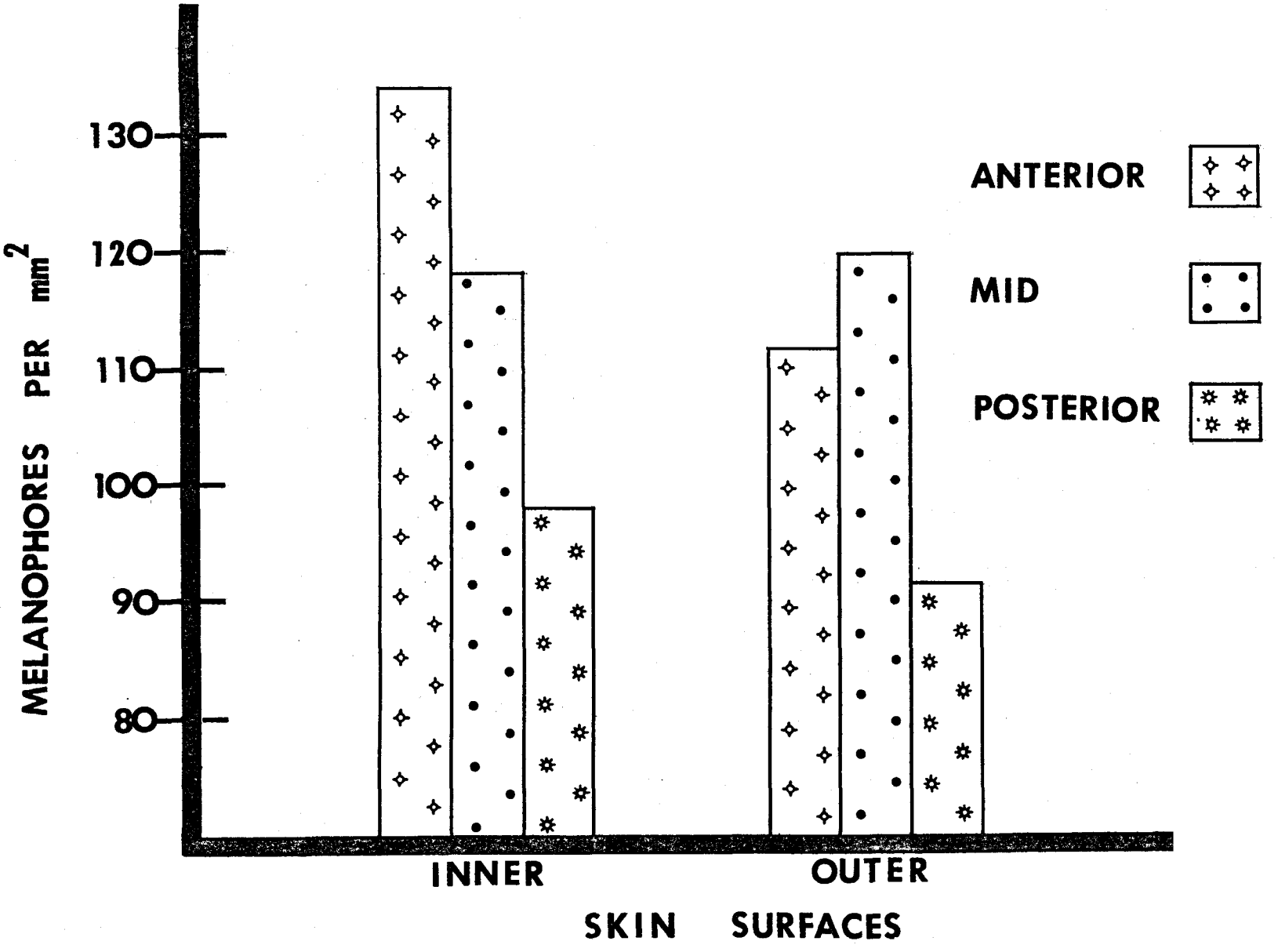

MELANOPHORES PER mm2
130
120
110
100
90
80
ANTERIOR
MID
POSTERIOR
INNER
OUTER
SKIN SURFACES

FIGURE 2. Histogram showing the average number of melanophores per mm^2 in the anterior, mid and posterior areas of the dark, intermediate and normal colored gar.

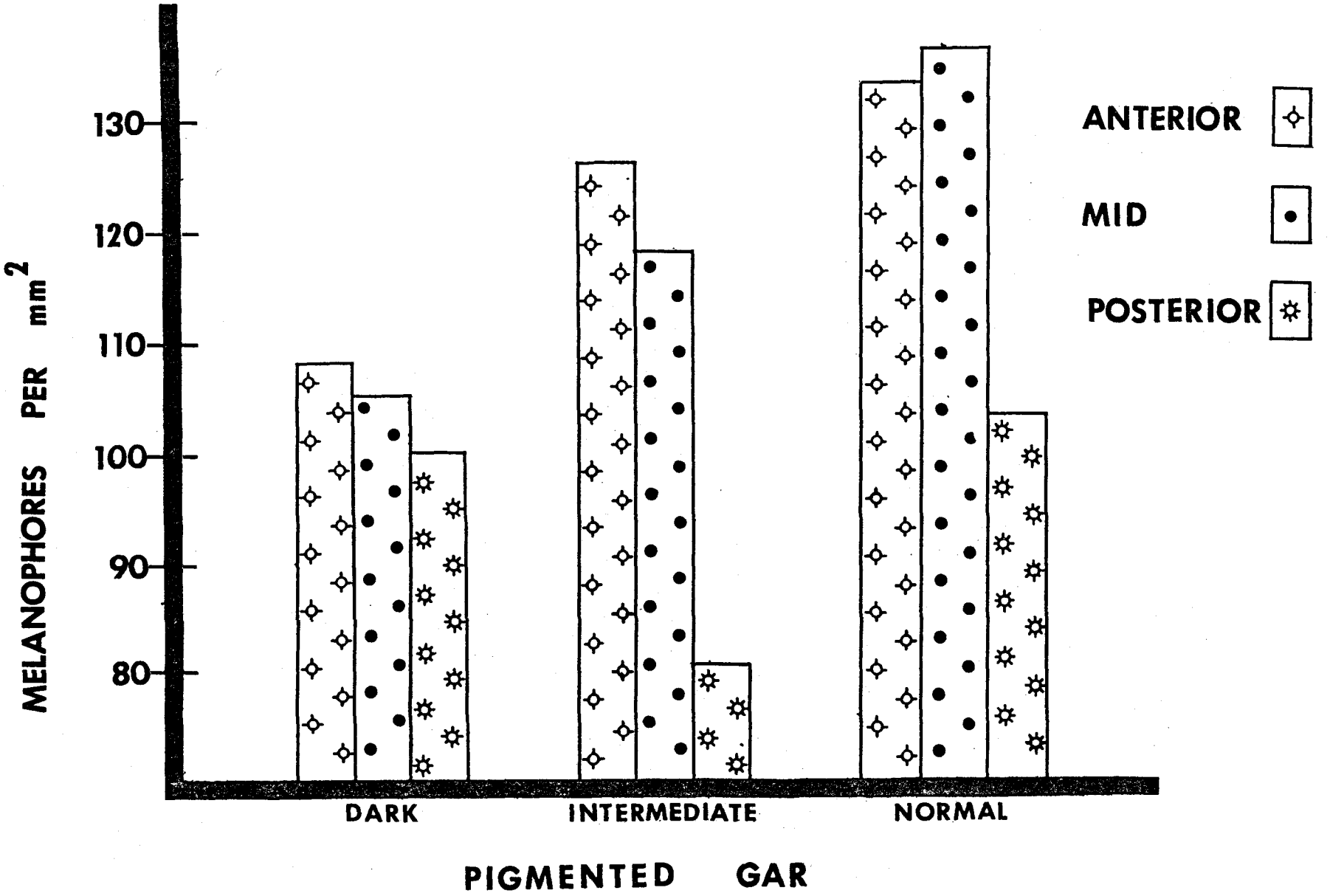

MELANOPHORES PER mm2
130
120
110
100
90
80
ANTERIOR
MID
POSTERIOR
DARK
INTERMEDIATE
NORMAL
PIGMENTED GAR

FIGURE 3. Reticulostellate melanophores from the mid dorsal outer skin surface of the normal gar at 33x.

FIGURE 4. Punctate melanophores from the mid dorsal outer skin surface of the dark gar at 400x.

FIGURE 5. Stellate melanophore from the anterior dorsal outer skin surface of the dark gar at 400x.

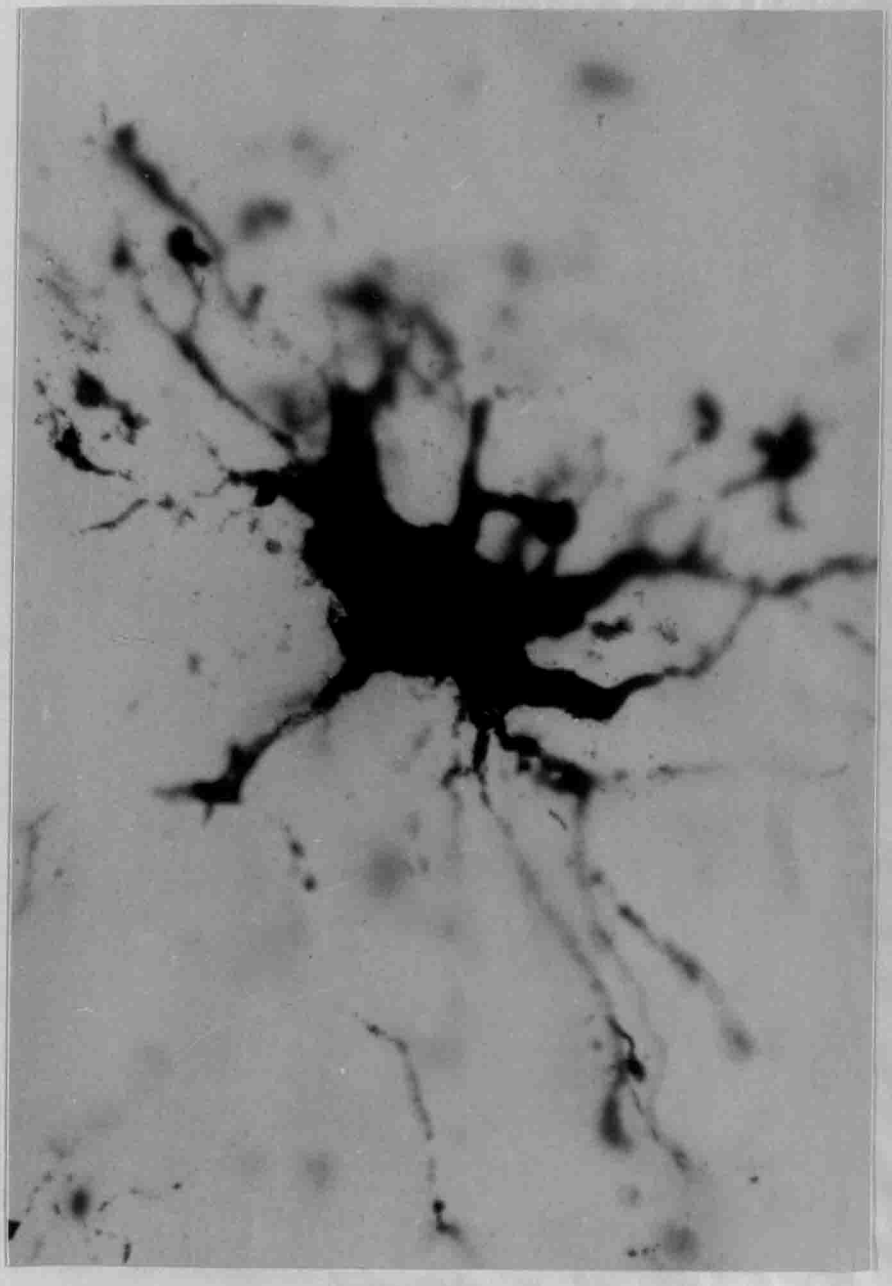


FIGURE 6. Stellate melanophores from the mid dorsal outer skin surface of the dark gar at 400x.

FIGURE 7. Reticulostellate melanophore from the anterior dorsal inner skin surface of the normal gar at 400x.

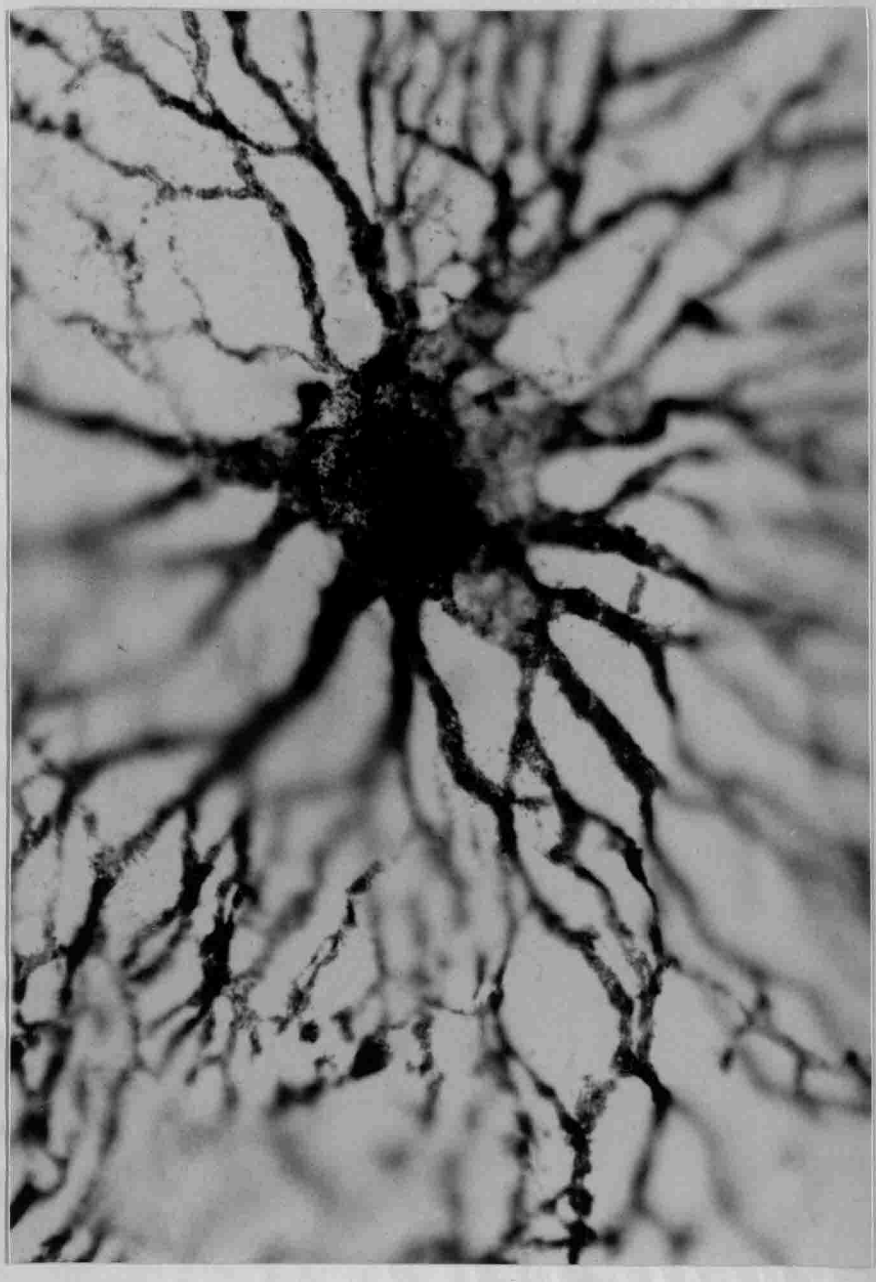


FIGURE 8. Reticulostellate melanophores from the mid dorsal outer skin surface of the dark gar at 85x.

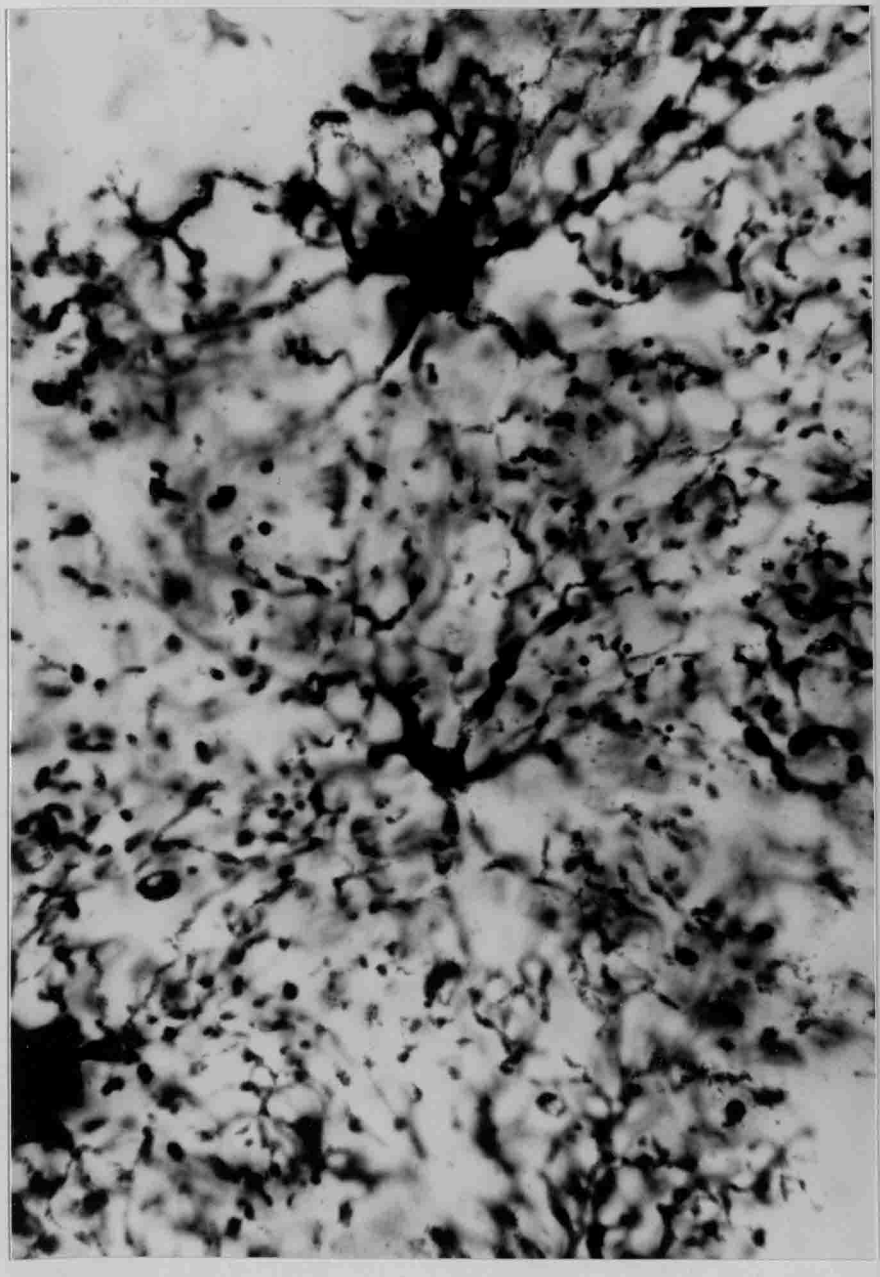


FIGURE 9. Reticulate melanophore from the anterior dorsal outer skin surface of the intermediate colored gar at 85x.

FIGURE 10. Reticulostellate melanophore from the posterior dorsal outer skin surface of the dark gar at 400x.

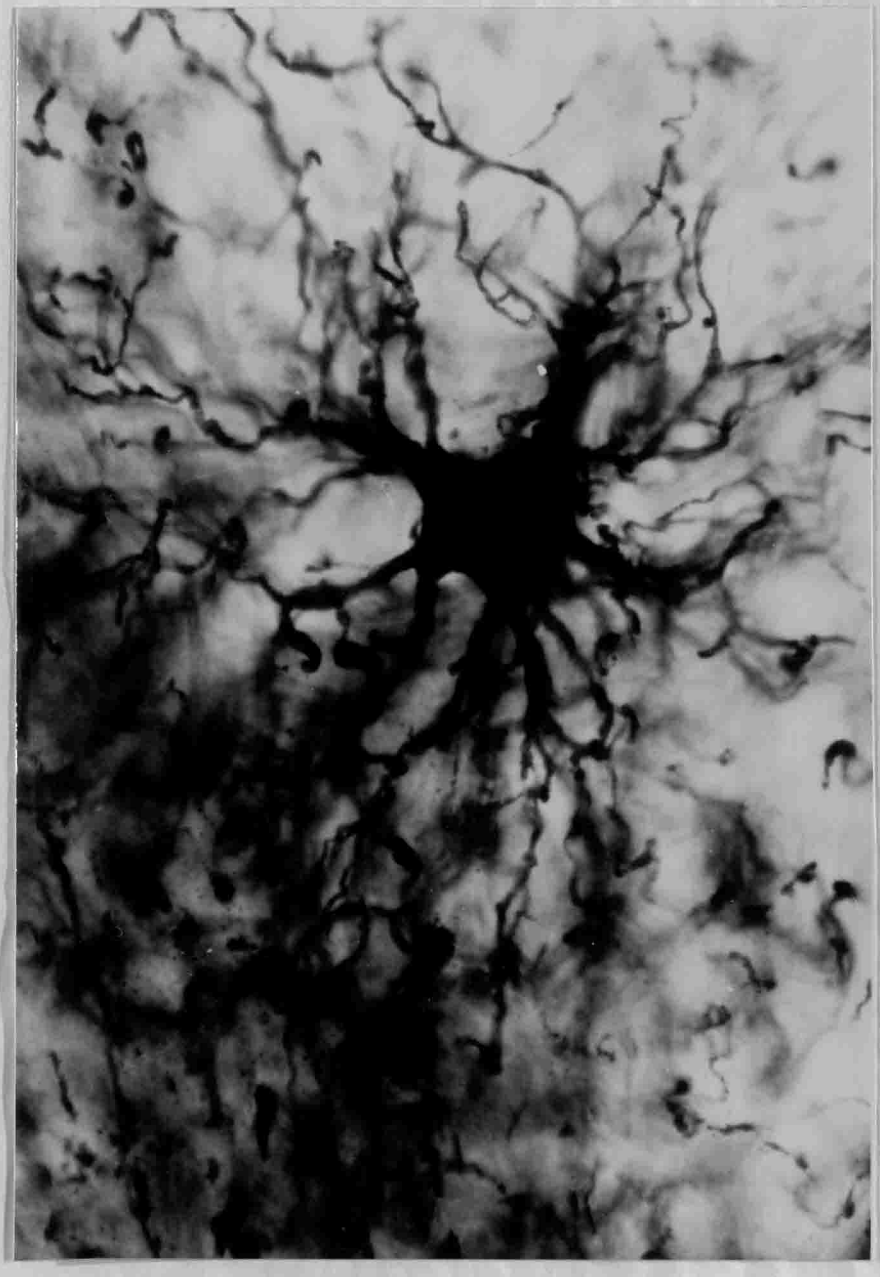
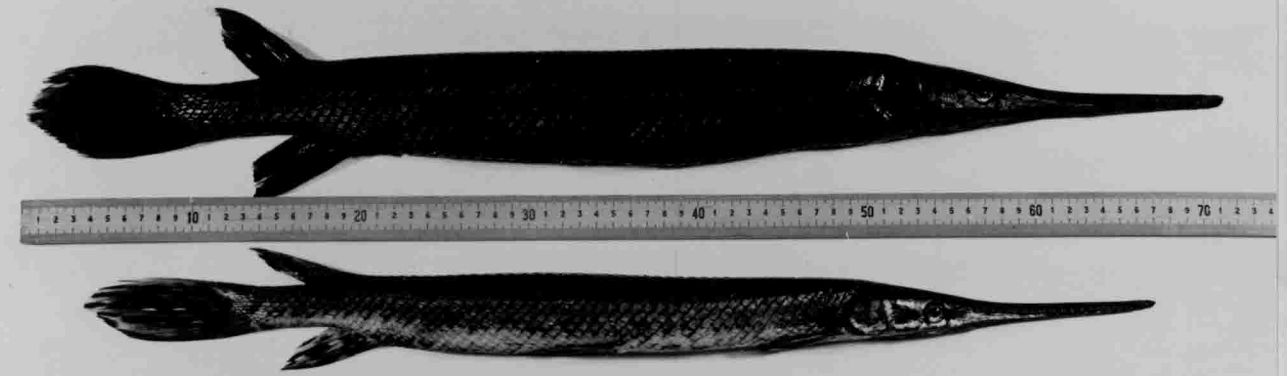


FIGURE 11. Photograph of dark and normal gar.



VITA

George William Minson, 2nd. was born in Petersburg, Virginia on August 10, 1949. He attended Flora M. Hill Elementary School, J. Mason Smith Junior High School, Colonial Heights High School and was graduated in June, 1967. He entered Virginia Commonwealth University in September, 1967, and was graduated in August of 1972 with a Bachelor of Science degree in Science. In August, 1972, he enrolled as a graduate student in the biology department of the University of Richmond and completed the requirements for the Master of Science degree in July, 1975.